ADA028199

USAMIIA TRANSLATION

Number: USAMIIA-K-5223

Date Completed: 1975

30 p.

Language: Russian

Geographic Area:

English Title: TOXICOLOGY OF TETRANITROMETHANE

Foreign Title: (Voprosy Promyshlennoy Toksikologii),

Author: A. I./Korbakova (edited by N. S. Pravdin)

Source Document: Trans. of Institute of Industrial Hygiene and Occupational Diseases, Problems of Industrial Toxicology (USSR) p208-238 1960.

Pages Translated: Pp. 208-238

Publisher:

Date/Place Publication: 1960, Moscow

D D C
RECEIVED
AUG 12 1976
B

409 112

INSTITUTE OF INDUSTRIAL HYGIENE AND OCCUPATIONAL DISEASES

PROBLEMS OF INDUSTRIAL TOXICOLOGY

edited by N. S. Pravdin

Moscow 1960

TOXICOLOGY OF TETRANITROMETHANE[*]

A. I. Korbakova

Tetranitromethane (TNM) is a new chemical compound widely used in industry. Based on experimental studies, ~~we~~ the authors determined two parameters of its toxicity, upper and lower, and established the zone of toxic action, an important factor in establishing the maximum permissible concentration of a new chemical substance in the air of work places. ~~We~~ They also tried to ascertain whether TNM can cause chronic poisoning by repeated exposure to low concentrations.

Review of the Literature
Physicochemical Properties of Tetranitromethane

TNM is a colorless, oily liquid with the characteristic odor of nitric acid. Its fumes are highly irritating to the mucous membranes of the eyes, nose, and upper respiratory tract. Its molecular weight is 196, specific gravity 1.650, vapor pressure at 20°C and at normal atmospheric pressure about 12mm Hg; highly volatile, volatility at room temperature 128mg/liter. Boiling point about 126°; it boils without breaking down, is not combustible and in pure form not explosive, but is highly explosive when mixed with carbon black and hydrocarbons. It freezes at about 13°, changing into acicular crystals. It is insoluble in water, but dissolves readily in ether or alcohol and is itself a good solvent for most organic compounds. It breaks down in an alkaline medium and when exposed to sunlight.

---

[*] An abridgment of a dissertation for the degree of candidate of medical sciences. Defended in January 1954.

The chemical structure of TNM is not completely clear, but many investigators are now inclining to the view that the TNM molecule $C(NO_2)_4$ has a symmetrical configuration.

The first cases of TNM poisoning were described by Fischer in 1917. They occurred in December 1916 in a war factory where TNT was melted and treated. Three workers -- a man and two women age 25 to 27 years -- suddenly became sick. They remained sick 6 to 14 days with similar symptoms. They complained of sluggishness, general weakness, and tightness in the chest. The skin and sclera of the two female workers were yellowish; the liver enlarged and tender. The symptoms gradually intensified and all three finally lapsed into unconsciousness and died.

In June 1917 at another war factory, 4 workers age 19, 23, 27, and 54 years were fatally poisoned. They were sick 4 to 7 months with complaints similar to the above.

Fischer encountered a few such cases in the chemical industry. The victims were 16 to 20 years old. Upon admission to the hospital, they complained of headaches, nausea, and vomiting. The symptoms intensified; they became unconscious and died. All developed jaundice.

Koelsche described similar cases of poisoning in 1917. Three fatal cases occurred in a factory manufacturing TNT. The workers in war factories where TNT was melted and treated complained of irritation of the mucous membranes of the nose, eyes, and upper respiratory tract, pressure pains in the chest, sluggishness, sleepiness, ready fatigability, and headaches. These complaints increased, as Fischer and Koelsche observed, when there was an especially disagreeable odor in the shop. Prolonged exposure intensified the headaches, pain in the heart, and restlessness. Speech incoherence was followed by a clouding of consciousness and death.

In all these fatal cases, gross inspection revealed yellowing of the skin and sclera, hair, and nails. At autopsy, the liver was generally reduced in size, solid, shriveled, rough on the surface and yellow in cross-section. The structure of the liver was indistinct, with pronounced fatty degeneration of the hepatic cells and numerous foci of necrosis.

In the United States, introduction of sulfite treatment of TNT helped to lessen the odor and, as Fischer noted, reduced the incidence and severity of poisonings.

The first experimental studies on the toxicity of TNM were run by Koelsche. His purpose was to determine the approximate concentrations of the poison that give rise to symptoms when inhaled, the possibility of the poison penetrating through the

skin, and its local effect when applied to the skin and when introduced into the stomach. Koelsche found that 1 or 2 drops on filter paper placed in the poisoning chamber markedly irritated the mucosa of cats and impaired their respiration. Four drops caused severe irritation and death 30 minutes after the animals were removed from the chamber. Ten drops caused even more severe irritation, nausea, and vomiting. The animals were removed from the chamber 10 minutes from the start of exposure and they died 15 minutes later.

Intragastric injection of cats with 15 drops of TNM in an alcohol solution killed the animals in a few days. Smaller doses, 1 to 5 drops, injected daily for 4 weeks resulted in a 30% weight loss and death.

The application of TNM in an oil solution to the skin did not result in any perceptible changes either at the application site or in the animal's general condition. However, subcutaneous injection quickly killed the animal.

All the animals that died after inhaling the fumes or following intragastric injection showed signs of irritation of the gastric mucosa, bronchopneumonia, pulmonary edema, swelling of the hepatic cells and, in addition, (after intragastric injection) fatty degeneration of the liver and kidneys.

Sievers et al. performed experiments on 11 cats with TNM of varying degrees of purity, including 2 experiments with purified wastes taken from boilers-traps. These experiments showed that TNM concentrations of 0.008 to 0.006mg/liter have only a slightly irritating effect on the mucosa of cats and no other clinical symptoms after two days of exposure, 6 hours each day. Concentrations of 0.06 to 0.6mg/liter perceptibly irritate the mucosa of the eyes, mouth, and upper respiratory tract and cause pulmonary edema and slight methemoglobinemia.

Histological examination of the animals' organs revealed a substantial serous exudate in the peribronchial fields of the lungs, infiltration of the bronchial mucosa with cellular elements, and a broad band of a serous exudate containing a few cells around the arteries.

The hepatic cells contained a moderate amount of fat. The epithelium of the convoluted tubules of the kidney also contained some fat and a few of the glomeruli were overflowing with blood. No appreciable changes were observed in the other organs.

M. Kiese's experiments also demonstrated the toxicity of TNM. Dogs, cats, and rats received intravenous, intramuscular, and subcutaneous injections of the poison. Kiese believes that regardless of the mode of entry, TNM is eliminated by the lungs, causing edema. He found pronounced liver involvement in all cases. He regards injury to the central nervous system as the main cause of death.

It is evident from this review of the literature that the toxicology of TNM has received very little attention. Clinical observations were confined to individuals who suffered from TNT contaminated by TNM. The experiments of Sievers et al. were performed not with pure TNM but with TNT of varying degrees of purity and with purified wastes collected in boilers-traps. Even concerning the degree of TNM toxicity we still do not have the amount of precise information needed to establish standards. Kiese's experiments cannot be used for this purpose because the animals were injected intravenously, intramuscularly, or subcutaneously. Since Sievers et al. performed their experiments with TNT contaminated by TNM, as mentioned above, their proposed maximum permissible concentration of 0.04mg/liter computed from the amount in TNT is highly doubtful, especially since Fercholl [?] suggests a permissible TNM standard lower by a factor of 50, i.e., 0.008mg/liter. However, he has offered no convincing justification for it.

Koesche's experiments provided only the qualitative characteristics of TNM as a toxic and highly irritating substance; they tell little about the quantitative aspects of its toxicity.

Thus, the task that we set for ourselves -- to evaluate the toxicity of TNM -- required us to perform appropriate experiments.

Determination of the Upper Parameter of Toxicity
Procedure

The upper parameter of TNM toxicity was investigated in 140 white mice weighing 18 to 20g previously observed for 10 days. The experiments were performed in a poisoning chamber (about 100-liter capacity) with an airtight cover. Twenty animals were used in each experiment with a given concentration of the compound. The mice were placed on the bottom of the chamber which was then hermetically sealed. TNM at the calculated dose was applied to filter paper with a pipet through an opening in the cover of the chamber. An electric mixer under the dome was used to hasten evaporation and ensure uniform distribution of the fumes. The exposure lasted 2 hours.

The concentration was calculated in mg/liter and verified by chemical analysis (Kuz'mina's method). To obtain very low concentrations, 0.1mg/liter or lower, the calculated amount of TNM was introduced into the chamber in a 1:100 alcohol solution. The concentrations mentioned below are the actual concentrations based on the results of chemical analysis.

The mice were withdrawn from the chamber after 2 hours of exposure and kept under observation for 24 days. Also

observed was the control, which was maintained under the same conditions as the experimental animals but not exposed to TNM.

The results of investigating the upper parameter of toxicity are summarized in Table 1. TNM at a concentration of lmg/liter killed most of the animals within a day or two of poisoning. At lower concentrations, most of the animals that survived the acute period died some time later. In view of this effect of the poison, we characterize the upper parameter of TNM toxicity from the death rate of the animals during the 24-day observation period.

(1) № опытов	(2) Концентрация мг/л	(3) Затравлено мышей	(4) Из них погибло (5) с указанием сроков гибели в сутках (6) в графе „0" показана гибель мышей во время ингаляционного отравления																									(7) Всего (8) погибло	(9) выжило	(10) смертность в %
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23			
1	5,0	20	20																								20	0	100
2	1,0	20	2	9		1	1			1		1	1	1	1	1	1	1									20	0	100
3	0,8	20		4				1			1	1	1				1								1	1	11	9	55
4	0,6	20		1		1		1				2								1	2						10	10	50
5	0,4	20				1	1		1							1			2				1				8	12	40
6	0,2	20														1			1				2	1			5	15	25
7	0,1	20																									0	20	0

Table 1. Death rate and survival time of white mice poisoned by the fumes of tetranitromethane at different concentrations.

Key: 1 - No. of experiment
     2 - Concentration, mg/liter
     3 - Mice poisoned
     4 - Number that died
     5 - Survival time in days
     6 - The column "O" shows the number of mice that died while inhaling the poison
     7 - Total
     8 - Died
     9 - Survived
     10 - Death rate, %

It is evident from Table 1 that the upper parameter of toxicity is limited to a very narrow range of concentrations,

from 0.1mg/liter (maximum tolerated concentration) to 1.0mg/ liter (absolutely lethal concentration). TNM is an extremely dangerous substance because increasing the maximum tolerated concentration to only 0.9mg/liter makes it absolutely lethal. The upper parameter of toxicity justifies our regarding TNM as a very toxic substance.

The clinical picture produced by poisoning mice was characterized chiefly by severe irritation of the mucosa and dyspnea. After exposure to low concentrations (below 0.1mg/ liter) the animals' behavior was indistinguishable from normal except for slight signs of impaired respiration (accelerated respiratory rate). After exposure to concentrations of 0.2 to 0.6mg/liter, signs of irritation of the mucosa quickly appeared: the animals sniffed all over the chamber, rubbed their muzzles with their paws, kept their eyes closed. Respiratory disturbances developed at the same time. The animals opened their mouths wide, breathing became hoarse, and a foamy blood discharge came out of their noses, and their breathing was clearly labored when the poisoning was terminated. Despite their poor condition, the animals tolerated the poisoning at the indicated concentrations and looked normal for several hours, with only slight dyspnea persisting for a while. However, a substantial number of the animals died in a few days.

After exposure to higher concentrations, 0.8 to 1.0mg/liter, irritation and dyspnea set in very quickly (within 15 minutes). Intense excitation and convulsions developed by the end of poisoning and the animals usually died the first day. The termination of poisoning did not improve the animals' condition. The respiratory disturbances intensified and the initial acceleration was followed by a slowing of the respiratory rate, and breathing became hoarse and labored. Finally, the animals died with signs of intense excitation and convulsions.

A similar picture was observed in rats and rabbits exposed to TNM fumes. The animals died 5 to 15 minutes after an intragastric injection of $0.02cm^3$ of TNM in the form of an emulsion in physiologic saline. Intense excitation was followed by convulsions and death.

Thus, the clinical picture of TNM poisoning is characterized by local irritation and excitation of the central nervous system manifested by initial accelerated respiration, general motor excitation, and convulsions. The symptoms of excitation were the same whether the animals inhaled the fumes or received the poison in the stomach.

In view of the presence of a nitro group in the TNM molecule, we made a spectroscopic determination of methemoglobin in the blood of the poisoned animals, but the results were negative.

-6-

Pathologoanatomical Changes

All the dead mice were dissected and examined in Professor P. P. Dvinkov's laboratory. Gross inspection revealed different changes, depending on the concentration of the poison and the time of death, but changes visible to the naked eye were particularly evident in the lungs of all the animals. Lung tissue was markedly hyperemic in the animals that died during poisoning. A foamy fluid sometimes exuded from an incision in the lungs and some fragments of lung were immersed in water. A small quantity of transparent fluid was generally found in the pleural cavity. The liver, kidneys, and spleen were hyperemic and the cerebral blood vessels dilated.

In animals that died at a later time, dissection of the thorax revealed multiple adhesions in the pleural cavity. The lungs were gray and there were pneumonic foci and sporadic areas of atelectasis. The liver was flaccid, pale, with an indistinct structure. The kidneys and spleen were hyperemic. Brain tissue was edematous and the blood vessels dilated.

Marked changes were evident in the animals that died 10 to 12 minutes after receiving an intragastric injection of TNM. The gastric mucosa was markedly hyperemic and had numerous petechial hemorrhages. Pulmonary tissue was likewise hyperemic and sometimes hemorrhagic. The changes were insignificant in the other organs. However, changes also occurred in other organs of the animals that died two or three days after receiving the poison in the stomach.

Histopathologic examination of the organs and tissues of the animals that died during poisoning in the chamber and the first few days thereafter revealed pronounced changes in the brain, mostly in the form of circulatory disorders and morphological alteration of the ganglion cells of the cortex and subcortex (Fig. 1). Circulatory disorders were also observed in the viscera in the form of edema, congestion; pulmonary edema was common. Characteristic changes were very often seen in the cells of the reticuloendothelial system in the liver. No particular changes, other than hyperemia, were observed in the other organs.

Figure 1.   Brain of a rat that died the first day after
            poisoning. Perivascular edema and perivascular
            hemorrhages.

        Protocol of the pathologoanatomical examination of mouse
No. 2 that died 24 hours after 2 hours' inhalation of TNM fumes
at a concentration of 0.8mg/liter.

        Lungs -- pronounced hyperemia, sporadic edema, epithe-
lium of some bronchi swollen, succulent.

        Liver -- swelling everywhere, occasional reproduction of
reticuloendothelial elements with the formation of small irregu-
larly shaped nodules from polymorphic cells.

        Kidneys -- hyperemia.

        Spleen and heart -- unchanged.

        Brain -- pronounced hyperemia, perivascular edema, oc-
casional small hemorrhages, acute swelling of cells of the
cerebral cortex, subcortex, and brain stem, a few vacuoles in
the protoplasm of cells in the subcortical region.

        In other cases of exposure to higher concentrations of
TNM, the central nervous system was more involved, but changes
in the viscera were less pronounced.

        If the animals experienced an acute period, they gen-
erally died later on, between days 10 and 20. Histological

-8-

examination revealed deeper involvement of the viscera, especially the lungs and liver as well as the cerebral cortex and subcortical region. The lungs usually showed signs of bronchopneumonia, edema, and in some cases abscesses. There were signs of bronchitis inflammatory or suppurative in nature (Fig. 2).

Figure 2. Lung of a mouse that died on the twelfth day after poisoning. Numerous edematous portions with pronounced reaction of histiocytic elements of the septa.

Distinct proliferation and marked swelling of the reticuloendothelial elements and occasional miliary necrobiotic foci were evident all over the liver (Fig. 3). In some cases, there

Figure 3. Liver of a mouse that died on the twelfth day. Miliary foci of necrobiosis.

was also a proliferation of the reticuloendothelium of the sinuses in the spleen.

The heart contained a large quantity of histiocytic elements in the interstitial tissue, sometimes directly under the endocardium.

Obvious changes were observed in the brain, which was usually very hyperemic, with glial elements accumulating around the blood vessels. Cells with swollen, translucent protoplasm were encountered in the cortex. Some cells exhibited dissolution of Nissl substance, vacuolation, and pyknosis. Distinct swelling of nerve cells in the subcortical region and vacuolation of their protoplasm were observed in the subcortical region. Swelling of the cells, vacuolation of the protoplasm, and, in places, lysis of the nuclei were also found in the brainstem.

Determination of the Lower Parameter of Toxicity

The conditioned reflex method has been used in Professor N. S. Pravdin's laboratory since 1964 to determine the threshold of injury caused by a chemical agent. The assumption is that the disruption of higher nervous activity by a foreign chemical substance is indicative of toxicity, even though other clinical symptoms of poisoning may be absent.

The concentrations of a chemical substance determined by the conditioned reflex method as being on the threshold of doing injury when compared with lethal concentrations of the same substance determine, as Pravdin pointed out, the zone of toxic action that characterizes the extent of the danger posed by the chemical.

The threshold of injury and zone of toxic action of a chemical compound, as determined by the conditioned reflex method, and its capacity for cumulative action (cf. below) serve as the experimental basis for recommending the maximum permissible concentration of the substance as a means of preventing it from harming the organism. Accordingly, we undertook to investigate the effect of TNM on the nervous system using the conditioned reflex method.

Method of Forming and Studying Conditioned Reflexes in Rats

We used the motor food method commonly employed in toxicological studies. The underlying principle is that a food motor reflex in response to sound and light is formed in an animal on the basis of an unconditioned food reflex.

Conditioned reflexes were formed and studied in rats in a special chamber (cf. O. G. Vasil'yeva's dissertation).

The process was started by extinguishing the animal's orienting reaction and the "what is this" reflex. The orienting reflex was extinguished as the animal became accustomed to the chamber and we started to form a positive conditioned reflex to the white light of an electric bulb using the technique employed in our laboratory.

At first the presentation of the conditioned (light) and unconditioned (food of "reinforcement") stimuli coincided in time, which was needed to form the conditioned reflex. However, when the latter was formed, presentation of the reinforcement "lagged" several seconds (10 seconds in our experiments) behind the stabilization of the formed reflex so that the conditioned stimulus kept acting for some time longer, but the animal did not receive any food even if it managed to run to the feed box. The reinforcement, always quantitatively and qualitatively the same (white bread in our experiments), was not supplied until after 10 seconds of isolated action of the stimulus.

The animals' behavior in the chamber varied. Some rats during stimulation and even reinforcement looked around the chamber in fright and did not take food, while others ate the food reinforcement calmly and still others carried the food off to a darker place where they ate it. Constant observation of the animals' behavior gave us preliminary impressions about their types of higher nervous activity which were then confirmed by the special tests described below.

After the formation and stabilization of the positive conditioned response to white light, we set out to establish differentiation. Differentiation was elaborated in the visual analyzer to blue light. When the chamber was illuminated with blue light, the rats did not receive reinforcement and therefore did not react with well-established differentiation to illumination of the feed box with blue light, remaining in their places. Complete ("zero") differentiation could not be established in a few animals. In such cases, the frequency of breakdown and speed of reaction to the negative stimulus was taken into account.

The parameters of higher nervous activity in normal animals and in those exposed to the toxic chemical were:

1. Latent period of the motor reaction, i.e., the time from the presentation of the conditioned stimulus to the start of the motor reaction in seconds.

2. Speed of the motor reaction, i.e., time required to run to the feed box in seconds.

3. Condition of the inhibitory process (differentiation) -- presence or absence of a motor reaction to blue light.

-11-

4. Successive inhibition -- absence or slowing of the motor
    reaction to the positive signal after presentation of the
    negative signal.

We thought it worthwhile for toxicological purposes to
add to the above parameters of higher nervous activity still
another one: appearance of phases in the activity of the cor-
tical cells under the influence of the poison. Accordingly,
after forming and stabilizing a conditioned reflex to a weak
stimulus like light in the rats, we formed a food motor con-
ditioned reflex in the same animals to a metronome (120 beats
a minute) as stronger physical stimulus.

Individual Characteristics of Higher Nervous Activity in the Experimental Animals

The main experiments performed by I. P. Pavlov's school
(A. O. Dolin, A. G. Ivanov-Smolenskiy, M. K. Petrova, Yu. P.
Frolov, and others) showed that the type of higher nervous ac-
tivity has a significant effect on the course of the pathological
process. Kotlyarevskiy, Gorsheleva, Izergina, Sanina, and
Kulagina also found that the individual characteristics of the
nervous system greatly influence the course of poisonings and
that weak animals tolerate them more poorly than do strong ones.

We base our description of the individual characteris-
tics of nervous activity in the experimental animals on the
main principles of Pavlov's doctrine regarding the types of
higher nervous activity. It is common knowledge that Pavlov
divided all animals into 4 main types according to the combina-
tion of properties of the nervous system: strength of
excitation and inhibition, degree of balance between the two
processes and their mobility.

1. Strong, balanced, labile animals -- lively, mobile type.
    Such animals quickly and stably form positive and inhibitory
    reflexes.

2. Strong, balanced animals with inert cortical processes --
    calm, slow type. They form positive and inhibitory con-
    ditioned connections with difficulty, which remain constant
    in magnitude and in time.

3. Strong, unbalanced animals with stimulation predominant --
    excitable type. Inhibition is comparatively weak because
    stimulation is dominant. Positive conditioned reflexes can
    be formed quickly and easily in such animals, but inhibitory
    ones are formed slowly and with great difficulty. It is
    sometimes impossible to form a stable and permanent in-
    hibitory reflex.

4. Weak, inhibited animals -- weak type. Quick inhibitability
    is characteristic of these animals. The slightest outside

-12-

stimulus deeply disrupts the conditioned reflexes, which are normally very unstable.

The following characteristics typify higher nervous activity in animals:

1. Rate of formation and stabilization of a positive conditioned reflex.

2. Nature of the positive reflex (the animal's behavior in response to a positive signal).

3. Effect of outside stimuli on the positive reflex.

4. Rate and nature of extinction and rate of subsequent restoration of the positive reflex.

5. Strength of the stimulatory process.

6. Rate of formation and stabilization of differentiation.

7. Stability of differentiation and fluctuations therein.

8. Strength of the inhibitory process.

9. Presence or absence of successive inhibition.

The strength of the stimulatory processes was tested by increasing the excitability of the food center through fasting. In animals with a strong stimulatory process, the positive conditioned reflex after being fasted a day increased or remained at the former level; in animals with a weak stimulatory process, the conditioned reflex decreased.

The strength of the inhibitory process was tested by prolonging the duration of the inhibitory (negative) stimulus. In the animals with a strong inhibitory process, prolongation of the stimulus to 3 minutes did not induce them to move to the feed box. "A strong cage can withstand it, but in a weak cage inhibition breaks down."[*] Strong but unbalanced animals cannot tolerate protracted inhibition because the inhibitory process is comparatively weak.

Successive inhibition, i.e., prolongation of the latent period or complete absence of a reaction to the positive stimulus after prolonged testing of differentiation, is an indication of slow concentration of inhibition, i.e., of weakness of the

---

[*]I. V. Pavlov. Pol. sobr. soch. (Complete Collected Writings), Vol. III, Book 2, p. 297.

inhibitory process. As training proceeds, active inhibition is usually concentrated more or less quickly so that successive inhibition disappears. If the inhibitory process is weak, concentration is slowed and at the same time successive inhibition is delayed.

The conditioned activity of the experimental animals under normal conditions and after exposure to TNM was recorded during the experiment and the diagrams shown below were constructed from the protocols.

Our experimental animals were distributed as follows: most of the rats (Nos. 2, 3, 5, 6, 7, 8, and 9) were regarded as having an unbalanced type of nervous system with the stimulatory process dominant and the others (rats Nos. 1 and 4) as having a weak type of nervous system in which both the stimulatory and inhibitory processes were weak.

Effect of TNM on Higher Nervous Activity in Rats

After stable conditioned reflexes were formed in the animals to the strong (metronome) and weak (white light) stimuli and differentiation to blue light, we set out to determine the threshold concentration of TNM from its effect on higher nervous activity.

The animals were exposed to different concentrations of TNM fumes in the same poisoning chamber that we used to determine the upper parameter of toxicity (cf. above). Poisoning was static and the concentration was established by calculations and checked by chemical analysis, as in the experiments on white mice exposed for 1 hour.

Before subjecting the animals to low concentrations of TNM, we ran some experiments in the poisoning chamber without the compound being present in order to exclude the influence of the new situation on the animals' conditioned activity. These preliminary control experiments did not appreciably alter the conditioned reflexes in some animals, but they did cause some disturbances in others. In the latter event, these experiments were repeated every day until the animal became accustomed to the new situation so that it had no effect on the conditioned reflexes. Only after we were convinced that the poisoning chamber had no effect on the reflexes did we begin to test the action of the TNM fumes on them.

The results of these experiments are shown in the diagrams below and in more detail in the corresponding protocols.

We tested TNM concentrations ranging from 0.002 to 0.01 mg/liter. A single exposure to 0.002mg/liter did not affect the

conditioned reflexes. The initial changes produced by a single
exposure were noted only after an 0.003mg/liter concentration
was used. The action of TNM was manifested here by an intensi-
fication of the inhibitory process (Fig. 4). In rat No. 2 under
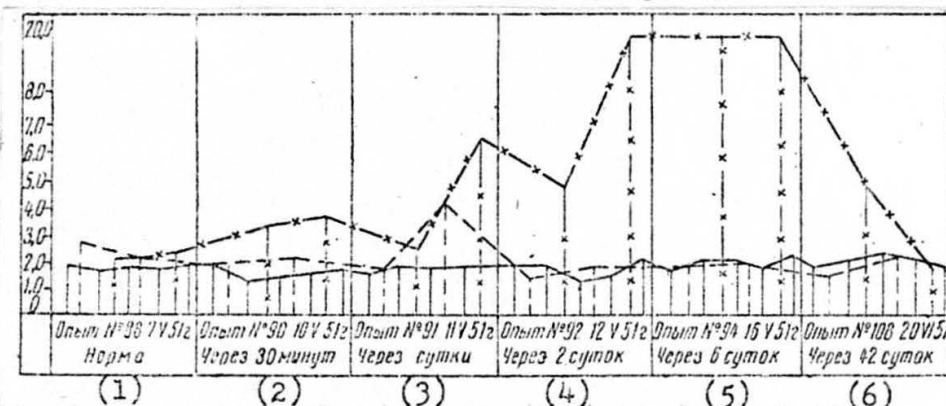

Figure 4.   Conditioned activity of rat No. 2 under normal
            conditions and after a one-hour exposure to TNM
            fumes at a concentration of 0.003mg/liter.

              1 - Experiment No. 88, 7 May 1951
                    Normal
              2 - Experiment No. 90, 10 May 1951
                    After 30 minutes
              3 - Experiment No. 91, 11 May 1951
                    After 24 hours
              4 - Experiment No. 92, 12 May 1951
                    After 2 days
              5 - Experiment No. 94, 16 May 1951
                    After 6 days
              6 - Experiment No. 108, 20 June 1951
                    After 42 days

normal conditions, differentiation was incomplete, but within
30 minutes of exposure inhibition became intensified as shown by
a slowing of the motor response to the differentiating stimulus
(higher ordinates above the differentiating signal). Inhibi-
tion intensified 24 and 48 hours later and after 6 days we observed
complete differentiation. It was not until 6 weeks later that
the conditioned activity of rat No. 2 returned to the baseline
level.

       The same concentration had a similar effect on another
rat.

       Thus, a TNM concentration of 0.003mg/liter under the
given experimental conditions caused initial disturbances of
higher nervous activity in animals and can therefore be regarded
as standing on the threshold of doing injury.

A further increase in the concentration to 0.005mg/liter caused in rat No. 4 (weak type) considerably disturbed both the excitatory and inhibitory processes for 2 months (Fig. 5). The
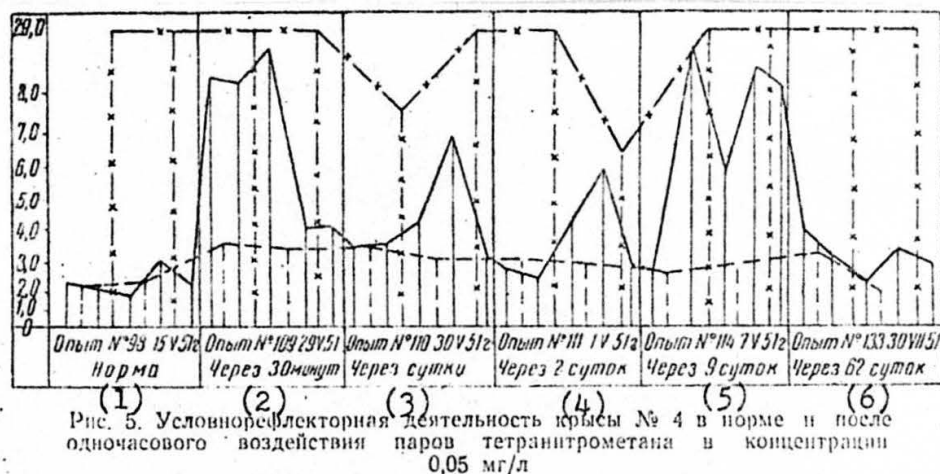

Рис. 5. Условнорефлекторная деятельность крысы № 4 в норме и после одночасового воздействия паров тетранитрометана в концентрации 0,05 мг/л

Figure 5.   Conditioned activity of rat No. 4 under normal
            conditions and after a one-hour exposure to TNM
            fumes at a concentration of 0.05mg/liter.

        1 - Experiment No. 98, 15 May 1951
                Normal
        2 - Experiment No. 109, 29 May 1951
                After 30 minutes
        3 - Experiment No. 110, 30 May 1951
                After 24 hours
        4 - Experiment No. 111, 1 May 1951
                After 2 days
        5 - Experiment No. 133, 30 July 1951
                After 62 days

disturbances, which extended to the point of complete loss of the positive conditioned reflex (experiment No. 114 and others) and which lasted an unusually long time, were due not only to the higher concentration of the compound but to the individual characteristics of higher nervous activity in the animal (weak type).

An 0.01mg/liter concentration was tested on 2 rats with different types of nervous systems. Rat No. 9, a strong, balanced animal, reacted to this concentration by developing protective inhibition in the cortex that was manifested by a loss of the positive conditioned reflex, drowsiness, and sleep (Fig. 6). In addition, inhibition also spread to the subcortical region and inhibited the unconditioned food reflex. The animal took the food reinforcement but did not eat it.
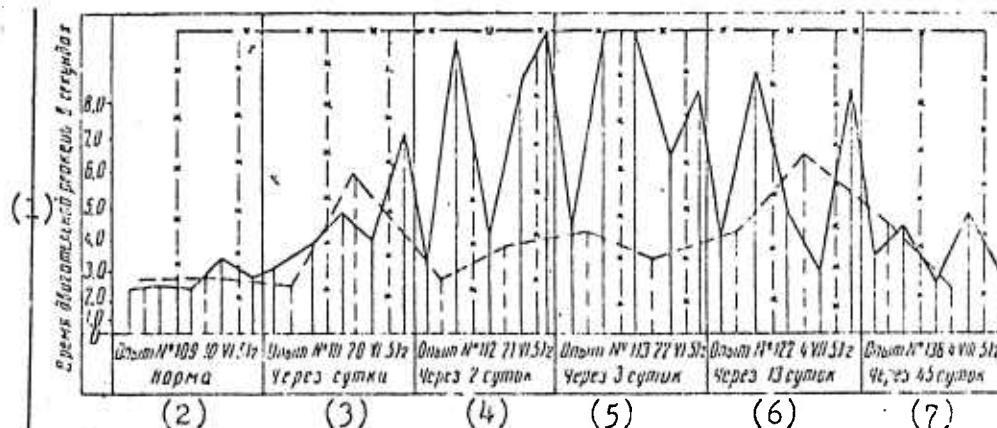
Figure 6. Conditioned activity of rat No. 9 under normal
conditions and after a one-hour exposure to TNM fumes
at a concentration of 0.001mg/liter.

1 - Time of motor reaction, in seconds
2 - Experiment No. 109, 10 July 1951
        Normal
3 - Experiment No. 111, 20 June 1951
        After 24 hours
4 - Experiment No. 112, 21 June 1951
        After 2 days
5 - Experiment No. 113, 22 June 1951
        After 3 days
6 - Experiment No. 122, 4 July 1951
        After 13 days
7 - Experiment No. 138, 4 August 1951
        After 45 days

Key: ———— Level of positive reactions to light
      - - - - Level of positive reactions to the metronome
      - x - Differentiations

Rat No. 1, an animal with a weak type of nervous system,
readily inhibited, with pronounced weakness of both the excita-
tory and inhibitory processes, exhibited a peculiar pattern of
disturbed conditioned reactivity. She did not react to the weak
stimulus (light), but continued to react to the strong stimulus
(metronome), although slowly. This state (narcotic phase) was
an indication of the onset of inhibition in the cerebral cortex
that was externally manifested by 3 days of sluggishness after
the poisoning, frequent sitting in the middle of the chamber and
not reacting to any stimuli, and sometimes falling asleep rolled
up in a ball. When she did react to the positive stimulus and
took the food placed in the feed box, she often did not eat it.
Inhibition spread to the subcortex, embracing the food center
and causing the unconditioned food reflex to become inhibited.
During the ensuing 75 days there were cyclic changes in the con-
ditioned reflexes and days with normal conditioned reflexes

-17-

alternated with days when they were impaired. It was not until 2 1/2 months after the single one-hour exposure to TNM fumes at a concentration of 0.01mg/liter that the animal's conditioned activity returned to normal.

These result of our study of higher nervous activity in rats using the conditioned reflex method show that minimum effective concentrations of TNM influence both the excitatory and especially inhibitory processes, intensifying the latter like bromine at all the concentrations tested. Low threshold concentrations intensified active internal inhibition and caused incomplete differentiation to become complete (rat No. 2, Fig. 5). The focus of inhibition arising in the cortex irradiated through the cortex. In doing so, its concentration slowed, as manifested by the appearance of successive inhibition. Higher concentrations produced protective inhibition, as manifested by the loss of conditioned reflexes, drowsiness, and sleep. Inhibition spread to the subcortical region, causing the unconditioned food reflex to become inhibited. The animal took food but did not eat it.

The above-described changes in conditioned activity persisted a long time. Restoration was slow and varied with the concentration of the compound and the individual characteristics of the animal's higher nervous activity. In rats with a weak type, the disturbances were more evident and restoration was unusually slow.

We also found 0.003mg/liter to be a threshold concentration. An 0.002mg/liter concentration did not produce noticeable changes in conditioned activity, but 0.005 and 0.01 mg/liter concentrations caused severe disturbances. Recovery of normal cortical activity took a long time.

Besides conditioned reflexes, we also used ergography to determine threshold concentrations of TNM. It was N. S. Pravdin who suggested that the threshold of injury caused by a poison be determined from its effect on muscular efficiency.

Most of the required apparatus (chamber and ergograph) consists of a combination of a small metal chamber (box) with a wooden stand in it for a frog and a special ergograph mounted in a small cabinet used to support the chamber (O. G. Vasil'yeva, 1951).

The experiments began with the recording of a normal ergogram for at least half an hour. Then, without interrupting the recording, the chamber was tightly closed with a wooden plate and air poisoned by the TNM fumes was passed through the chamber. The experiments continued until some effect was observed, after which, still without interrupting the recording,

a gosling [?] was removed from the chamber to determine the course of restoration of muscular efficiency impaired by the poison. The effective concentration was determined by dividing the amount of substance expended (from the weight loss of the gosling) by the amount of air passed through the chamber during the action of the poison as determined with a rheometer. The result was checked by chemical analysis.

The effect of TNM on muscular efficiency as judged from the results of the experiments are shown in Fig. 7 in the
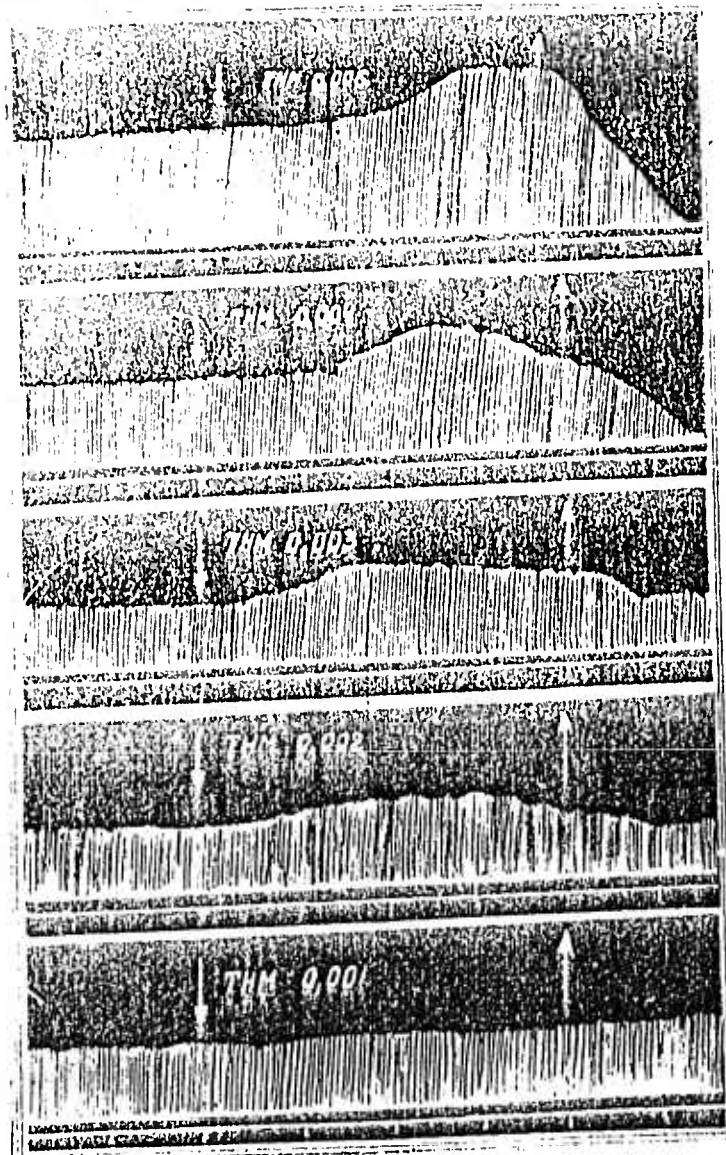

Figure 7. Series of ergograms of the gastrocnemius muscle of a decerebrated frog, with the blood supply retained, after exposure to different concentrations of TNM fumes. Up to the arrow pointing upward -- normal. Between the arrows -- effect of the poison. Arrow pointing downward -- exposure to the poison halted.

Time mark -- every 15 minutes.

series of ergograms of frogs exposed to different concentrations ranging from a maximum of 0.006 to 0.001mg/liter. Each concentration was tested on at least 3 frogs and a series of the most characteristic ergograms was chosen from all the material obtained.

It is evident from the figure that the effect of the TNM fumes on a whole frog with a destroyed central nervous system and retained blood supply was manifested by as clear an impairment of muscular efficiency as the disturbance of higher nervous activity in the rats exposed to the same concentrations of the poison.

We concluded from the ergographic studies that a TNM concentration of the order of 0.002 to 0.003mg/liter is the threshold of injurious action. These concentrations, obtained by 2 different methods of investigation, characterize the lower parameter of TNM toxicity.

Change in Reactivity of Animals after Repeated Exposure to Tetranitromethane

We used our routine laboratory method to determine the effect of repeated exposure to the poison.

Sixty mice weighing about 18g were divided into 2 groups of 30 animals in each. The "experimental" group was exposed to 2 hours of TNM fumes at a concentration of 0.05mg/liter daily for 24 days, a dose one-tenth the median lethal concentration of 0.6mg/liter (established for mice, which killed half the poisoned animals (Table 1). The "control" group was kept under identical conditions but not exposed to the poison. None of the control or experimental animals died throughout this 24-hour period and none exhibited any pathological phenomena. After 24 days, both groups were placed together in a common chamber and exposed for 2 hours to the previously established median lethal concentration of TNM, i.e., 0.6mg/liter. We expected more experimental than control mice to die in the event of functional cumulation of the effect. In fact, however, the opposite happened: fewer experimental animals died and they did so later than the control (Table 2).

-20-

(1) Группы	(2) Концентрация мг/л	(3) Затравлено мышей	(4) Из них погибло (5) с указанием дня гибели после завтрака																							(6) Всего		(9) Смертность в %
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	(7) погибло	(8) выжило	
(10) Опытная (предварительно многократно отравлена)	0,6	30	—	—	—	—	—	—	—	2	—	2	1	—	—	1	1	—	—	—	—	—	—	—	—	—	7	23	23
(11) Контр. (отравл. впервые)	0,6	30	10	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	13	17	43

Table 2. Death rate of white mice exposed to 2 hours of TNM fumes at a median lethal concentration of 0.6mg/liter after repeated exposures to a subtoxic concentration of 0.05mg/liter.

Key:
1 - Groups
2 - Concentration, mg/liter
3 - Number of mice poisoned
4 - Number of those killed
5 - Day of death after poisoning
6 - Total
7 - Died
8 - Survived
9 - Death rate, %
10 - Experimental (repeated previous poisoning)
11 - Control (poisoned for the first time)

    The clinical picture in the experimental group was also different from that in the control. The differences were particularly pronounced after exposure. The control animals lay motionless the first few hours; their respiration was labored and hoarse. The experimental animals were more lively and their respiration was less disturbed. One had the impression that the experimental mice had become "habituated" to TNM.

    Microscopic examination of the organs of the experimental animals revealed pronounced changes in the cells of the cortex, subcortex, and brainstem, indicating that the apparent "habituation" was accompanied by a distinct pathology.

    To confirm this unexpected result, we ran a second experiment in which the indicator of the cumulative effect was to be the state of higher nervous activity as determined by the conditioned reflex method rather than the death rate.

Three rats (Nos. 2, 4, 9) previously used to determine the threshold concentrations were the experimental animals. As already indicated, they were exposed once to 3 different TNM concentrations, 0.003, 0.005, and 0.1mg/liter, respectively, and a definite conditioned effect was determined for them (Figs. 4, 5, and 6) and adopted as the baseline for purposes of comparison with the results of exposure to subthreshold concentrations.

Some time (about 3 months) after the original conditioned activity was completely restored in these animals, they were exposed daily to 2 hours of TNM fumes at a concentration of 0.005mg/liter for 2 weeks. Then each of the 3 rats was separately exposed for one hour to the same concentration as 3 months before, i.e., 0.003, 0.005, and 0.01mg/liter, respectively. It turned out that the effect on conditioned activity in rat No. 2, which was exposed to the lowest concentration (0.003mg/liter), was as before, but restoration occurred sooner. The effect was less pronounced in rats Nos. 4 and 9 and restoration occurred much sooner.

Thus, in this experiment too, instead of the expected cumulative effect we found "habituation." Could this "habituation" be regarded as a positive phenomenon? We shall try to answer the question in the discussion of the results.

We also observed adaptation to repeated exposures when rats were subjected for one hour every day for 20 days to TNM fumes at the initially tested concentration of 0.02mg/liter, which is essentially a subthreshold concentration.

Here "habituation" was manifested by the fact that the positive conditioned reflexes which weakened during the first half of the 20-day experimental period became normal during the second half and remained normal until the end of the experiment, despite the continued daily exposure.

Regarding the inhibitory process in the rats, it was impossible to detect any "habituation" at least during the 20-day experimental period. Two rats with complete and stable differentiation suffered breakdowns (disinhibition), which ceased as soon as the daily poisoning was halted. No changes in the nature of the inhibitory process were observed in the rats with incomplete differentiation.

Local Exposure

The tails of white mice protected against the inhalation of TNM fumes were forced into a test tube with liquid TNM and kept there for an hour. No pathological changes were noted in the skin of the tails either immediately after they

were withdrawn from the liquid or for 3 weeks thereafter.
Nor were there any symptoms of systemic poisoning that would
have implied the penetration of TNM through the skin.

A glass shell [?] with an opening closed with a
ground glass stopper was glued to a round spot 4 to 5cm in
diameter on the shorn and shaved side of a rabbit and fixed
with a bandage. Two to 3ml of TNM was poured into the shell
and it spread over the shaven surface of the skin and re-
mained in contact with it 1/2 to 2 hours. After the shell
was removed, no pathological changes were seen at the appli-
cation site of TNM for so long as no abrasions or scratches
were inflicted during the shearing and shaving. If there
were, the site became very red and inflamed with edema of
the underlying tissue, sometimes with the formation of an
eschar. When the latter was sloughed off, the resulting ulcer
healed slowly.

Injection of 1 or 2 drops of TNM into the eye of a
rabbit provoked a sharp motor reaction. The conjunctive be-
came red in 10 to 20 minutes. The eye closed and could not
be opened without difficulty; the corneal reflex was absent.
The cornea became rough in a few hours and the next day it
was found to be completely opaque. The inflammation subsided
in 1 to 1 1/2 months, but the opacity remained.

These experiments showed that TNM probably does not
act on intact skin and apparently cannot penetrate it in
amounts sufficient to induce poisoning. However, it is highly
irritating when it strikes injured skin. It is particularly
dangerous when it gets into the eyes.

DISCUSSION

Our studies on the toxicity of TNM give some idea of
the general nature of the effect of the poison on animals.
Specifically, we determined the main parameters of toxicity
that are needed to set standards for the content of TNM fumes
in the air of work places, lethal and threshold concentra-
tions. We also learned the effect of repeated exposures to
subthreshold concentrations and found morphological changes
in the nerve cells of the brain and other organs of poisoned
animals.

All these findings indicate that TNM is highly toxic
because a slight increase in minimum concentrations can easily
make them lethal owing to the narrow zone of toxic action.

The high toxicity of TNM is perhaps largely due to
the fact that it is not only pronounced a local irritant but
also possesses resorptive action. Our studies on conditioned

reflexes showed that even after inhalation of concentrations
as low as 0.01mg/liter, the cortex experiences protective in-
hibition. The strength of this inhibitory process can be
assessed because it is not limited to the cerebral cortex
but irradiates to the subcortex, resulting in impairment of
the unconditioned food reflex.

In view of the described properties of TNM, it is
easy to see why by acting on the extero- and interoreceptors
it severely disturbs the blood supply, thereby inhibiting the
"chemical vital process" in the cortical nerve cells and pre-
venting them from resisting the various destructive influences
constantly at work outside and inside the organism. TNM cir-
culating in the blood gives rise to a pathological process in
functionally weakened nerve cells that disables them and thus
interferes with the regulatory function of the cerebral cortex.

It is common knowledge that the outcome of a poisoning
depends not only on the poison and conditions of its action
but also on the functional state of the organism at the time
of the poisoning, particularly, the functional state of the
nervous system. Animals with the strong type of higher ner-
vous activity are known to tolerate poisoning more readily
and survive more often than animals with a weak type. Pre-
sumably, the animals that tolerate and survive poisoning by
median and higher lethal concentrations of the poisoning have
the strong type of higher nervous activity. They also remain
capable of resisting other kinds of destructive influences,
e.g., infectious pneumonia combined with poisoning against a
background of irritation of lung tissue. In other words,
animals that can tolerate poisoning by high concentrations
usually survive the immediate post-intoxication period. Our
experimental mice that survived poisoning by high concentrations
of TNM when poisoned again by TNM often died much later. The
mice apparently died because a single exposure to the com-
pound resulted in disruption by reflex action of the trophic
relations between the nerve cells of the cerebral cortex and
induced a pathological process, which proved to be the cause
of the later death of animals that lost the ability to resist
injurious factors.

The lower parameter of TNM toxicity that we determined
from 2 threshold indicators -- disruption of conditioned ac-
tivity and impairment of muscular efficiency -- did not provide
a conclusive answer to the question of the threshold of in-
jurious action of TNM for man, even though both indicators
produced similar results, especially since we have no data for
judging the possibility of chronic poisoning arising and deter-
mining the conditions under which it develops. Our experiments
showed that insignificant subthreshold concentrations of about
0.0005mg/liter (5 to 6 times below the threshold concentration)
with repeated exposures, judging from their effect on conditioned

-24-

activity, not only does not result in functional cumulation but even increases the animals' resistance to the poison, as shown by smaller changes in the conditioned reflexes. Repeated exposures to higher concentrations of about 0.05 mg/liter likewise do not result in functional cumulation. Rather, they increase the animals' resistance to still higher lethal concentrations.

We ascribe the phenomenon of habituation chiefly to the great plasticity of the nervous system. In his famous "A Physiologist Answers the Psychologists," Pavlov wrote:

> The most important, most powerful, and most enduring impression obtained from studying higher nervous activity with our method is the extreme plasticity of this activity, its vast capabilities: nothing remains immovable or unyielding, but everything can be achieved and made to change for the better provided that the appropriate conditions be created.

These words of the great physiologist reflect his deep conviction that by virtue of its extreme plasticity, any "alteration" of the nervous system is possible, including change in the innate type of higher nervous activity. The plasticity of the nervous system was studied by Pavlov's co-workers and students and demonstrated in an excitable dog in which differentiation was formed. The "alteration" method was very often used for this purpose, when a positive stimulus was not reinforced and there should have been inhibition, while an inhibitory stimulus accompanied by reinforcement should have elicited a positive response. Usiyevich was able to induce an alteration three times in a dog. In doing so, the positive effect increased with each alteration but less time was required to bring about the alteration. Such alteration appears to be essentially little more than the result of training.

Training the nervous processes, as G. V. Fol'bort and his co-workers discovered, follows certain laws that seem to be common to all functioning organs. One of them is that the

> return of an exhausted organ to normal entails two different processes: restoration in the narrow sense of the word, or increase in functional potential, and stabilization of the state of restoration achieved.

In connection with these laws, Fol'bort advanced, in passing, a view that reveals, we believe, the great opportunities provided by toxicological studies for explaining the mechanism of habituation to poisons and the mechanism of poisoning from the standpoint of Pavlovian nervism. Fol'bort said:

Finally, I must also point out that two opposite states may develop after repeated functional stresses. If each succeeding stress comes from a normal stabilized state of the organ, the functional potential of the organ, its work capacity, increases perceptibly, mainly because the process of restoration is accelerated. This is obviously the well-known process of training. However, if a new stress is placed on the organ whose restoration is not yet stabilized, the restoration process is slowed and weakened and the organ will become chronically exhausted.

Compensation develops after poisoning, i.e., the balancing of the disturbances produced by the poison, and if the compensation is adequate, we may not even notice the disturbances produced by the poison. For example, a poison can impair the tissue respiration of some organ while the oxygen requirement of the integral organism remains normal. But during this time the cerebrocortical cells, which "must be acknowledged as being highly reactive and, consequently, destructible," have suffered definite injury and "restoration of the expended irritable substance" is essential if they are to continue to function."[*]

If the expended irritable substance is restored and the restoration is stabilized before a new functional stress of the same magnitude is placed on the cerebral cortex and repeated in the same rhythm, the cortical cells will not only continue to function normally but also become trained and, as a result, be better able to tolerate the effects of a poison, as we observed in our experiments with the cumulative effects of TNM.

These experiments confirmed Pavlov's views on the extreme plasticity of higher nervous activity. In fact, we saw adaptation, a balancing of the organism, even though there were pronounced morphological changes in the cells of the cortex, subcortex, and brainstem. However, owing to the great compensatory capabilities of the organism, functional changes had not yet taken place. We will probably see the limit of adaptation to a poison when the nervous system enters a state of chronic exhaustion. This will most likely be chronic poisoning. "Habituation" to a poison is presumably one of the stages of chronic poisoning.

CONCLUSIONS

1. The lethal concentration of tetranitromethane (TNM) for white mice is 1.0mg/liter.

---

[*] I. P. Pavlov. Poln. sobr. soch. (Complete Collected Writings), 1951, Vol. 3, Book 2, pp. 61 and

2. The minimum active concentrations capable of disrupting conditioned reflexes in rats and altering muscular efficiency are of the order of 0.003mg/liter.

3. The effect of TNM is manifested by marked irritation of the mucous membranes and signs of excitation of the central nervous system -- irregular respiration, general motor excitement, and convulsions which result in death.

4. The morphological changes appearing in the organs and tissues of the animals killed by TNM are characterized by pronounced vascular disorders in the form of hyperemia of all the internal organs and especially the brain.

5. In view of the marked irritating effect of TNM when it strikes the eyes, appropriate precautions should be taken when working with it.

6. Prolonged, repeated exposure to subthreshold concentrations of TNM can cause pathological changes in organs and tissues.

7. Because of the narrow zone of toxic action, steep rise in the mortality curve, low threshold of injury (0.002 to 0.003mg/liter), and the ability of TNM to produce pathological effects, we recommend a maximum permissible concentration of about 0.001 to 0.0003mg/liter of air.

8. The "habituation" that we observed in repeated experiments is an indication of temporary compensation of the effects of the poison.

It would be worthwhile in future research to study the duration of the compensation period as well as the functioning of various organs of animals in the compensation stage.

BIBLIOGRAPHY

1. Vasil'yeva, O. G., 1951, Contribution to the Toxicology of Diethylamine. Dissertation (manuscript), Moscow.

2. Ivanov-Smolenskiy, A. G.,1949, Ocherki patofiziologii vysshey nervnoy deyatel'nosti (Outline of the Pathophysiology of Higher Nervous Activity), Medgiz.

3. Lazarev, N. V., 1938, Osnovy promyshlennoy toksikologii (Principles of Industrial Toxicology, Medgiz.

4. Pavlov, I. P., Polnoye sobraniye sochineniy (Complete Collected Writings), Vol. 3, Book 2.

5. Petrova, M. K., 1946, *O roli funktsional'no oslablennoy kory golovnogo mozga v vozniknovenii razlichnykh pato-logicheskikh protsessiv v organizme* (Role of Functional Weakening of the Cerebral Cortex in the Development of Various Pathological Processes), Medgiz, Moscow.

6. Folbort, G. V., 1951, "New Facts and Considerations Relevant to I. P. Pavlov's Theory," etc., *Zhurn. vyssh. nervn. deyat* (Journal of Higher Nervous Activity), 3.

7. Frolov, Yu. P., 1944, *Vysshaya nervnaya detatel'nost' pri toksikozakh* (Higher Nervous Activity in Toxicoses), Medgiz.

8. Koelsche, F., 1917, "Die Giftwirkung des Tetranitromethan," Zbl. f. *Gewerbehygiene*, V, 185.

9. Fischer, 1917, "Tödliche gewerbliche Vergiftungen durch Trinitrotoluol und Tetranitrmethan," Zbl. f. *Gewerbehyg.*, V.

10. Sievers, R. F. and [text illegible], 1947, "Toxic Effect of Tetranitromethane," Public Health Service, *Pub. Health Report* 62:2798.

11. Kiese, M., 1949, "Zur Pharmacologie des Tetranitromethan," Arch. f. exp. *Path. und Pharm.*, V-VI:556.